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Natural Killer Cells Induce Activated Murine B Cells to Secrete Ig[1]

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ABSTRACT. We previously demonstrated that dextran-conjugated anti-IgD antibodies (αδ-dex) induce proliferation of small, B cell-enriched murine spleen cells (B^e cells), and in the presence of IL-2, stimulate Ig secretion in vitro. We have shown that αδ-dex-stimulated B cells provide an in vitro model for studying B cell activation by T cell-independent type 2 (TI-2) Ag, as exemplified by the bacterial polysaccharides. We now show that highly purified resting B cells, obtained by electronic cell sorting (B^{sp} cells), fail to secrete Ig in the presence of αδ-dex + IL-2. The αδ-dex + IL-2-induced Ig secretory response of B^{sp} cells is restored upon addition of splenic non-B, non-T cells or a pure population of in vitro-generated NK cells. Similarly, pretreatment of B^e cells with anti-AsGm-1 plus complement inhibits Ig secretion in response to αδ-dex + IL-2. An IL-2-induced NK cell supernatant (NKSN) is equally potent at stimulating Ig secretion by αδ-dex-activated B^{sp} cells, indicating that cell contact between B^{sp} and activated NK cells is not required for this effect. IL-2 stimulates not only NK cells, but B cells as well, since addition of anti-IL-2 + anti-IL-2R antibodies to B^{sp} cell cultures, in the presence of αδ-dex + NKSN, inhibits Ig secretion. These data describe a novel animal model for NK cell-induced B cell maturation to Ig secretion and suggest a pathway for Ig production in response to TI-2 Ag. *Journal of Immunology*, 1993, 151: 5251.

The parameters that regulate Ig production in response to TI-2 Ag are largely unknown. Such Ag, as exemplified by the bacterial polysaccharides or certain autoantigens, such as DNA, are characterized by multiple repeating antigenic epitopes, conferring upon the Ag an ability to extensively cross-link Ag receptors on the surface of B cells and hence induce activation (1–3). That TI pathways for induction of Ig secretion exist, is supported by the observation that T cell-deficient nude mice synthesize IgM and IgG antibodies in response to TI stimuli (4) and by the finding of normal serum levels of IgG3, IgG2b, and IgG2a, and undiminished antibody responses to TI-2 Ag, in mice made genetically deficient in MHC class II expression, and thus non-responsive to T cell-dependent (TD) Ag (5). In vitro studies have demonstrated that while TI-2 Ag could stimulate Ig secretory responses in resting B cells in the absence of T cells, some form of cytokine-mediated help was required, and in its absence no responses were observed (6). This suggested that in vivo responses to this class of Ag might also require some form of ancillary non-T cell-derived help. A role for such help is also suggested by the ability of TI-2 Ag to stimulate Ig class switching (4, 7). In this regard it has been demonstrated that IFN-γ, released by activated NK cells (8), in addition to T cells, induces IgG3 (9) and IgG2a (10) class switching in the mouse. In vivo studies indicate that NK cells secrete IFN-γ early during the course of bacterial infections, without a requirement for T cell help (11, 12). Further, in vitro studies demonstrate that NK cells can provide a source of

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IFN-γ for induction of IgG2a class switching by LPS-activated B cells (13, 14).

We recently described an in vitro polyclonal system for studying B cell activation in response to TI-2 Ag (6, 15). Multiple anti-IgD antibodies were covalently linked to a high molecular weight dextran (αδ-dex)[3] to simulate the repeating epitope nature of polysaccharide Ag. Small, B cell-enriched murine spleen cells (B^e cells) proliferated in response to αδ-dex and secreted Ig upon addition of IL-5 or IL-2 (6). By contrast αδ-dex induced large, low density B cells to secrete Ig in the absence of exogenous cytokines. These responses were similar to in vitro TNP-specific Ig responses stimulated by TNP-Ficoll, a prototypic TI-2 Ag. Given the small numbers of Ag-specific B cells in the unimmunized mouse, the advantages of αδ-dex lies in its ability to polyclonally activate the vast majority of mature B cells through the membrane (m)Ig pathway in a TI-2-dependent fashion and thus provide a model to study parameters regulating these responses.

In previous studies utilizing αδ-dex, we employed a population of small T-depleted spleen cells that were highly enriched for B cells (90 to 93%). More recently we obtained highly purified (>98%) $mIgM^+$ B cells (B^{sp} cells) by electronic cell sorting to determine whether IL-5 and IL-2 acted directly on αδ-dex-activated small B cells to induce Ig secretion. We observed that small B^{sp} cells showed a dramatically reduced Ig secretory response to αδ-dex + IL-2, while synthesizing comparable amounts of Ig, relative to B^e cells, upon stimulation with αδ-dex + IL-5 or LPS. This suggested that, at least for responses to αδ-dex + IL-2, a non-B, non-T cell, which was not present in the sort-purified population, was required. On the basis of this observation, we conducted further experiments which demonstrated that murine NK cells could stimulate B cells to mature to Ig secretion. This establishes a murine model for investigating the cellular basis for TI-mediated humoral immune responses.

Materials and Methods

Mice

Female DBA/2 and BALB/c mice were obtained from the National Cancer Institute (Frederick, MD) and were used at 7 to 10 wk of age. The experiments were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Animal Resources, National Research Council, Department of Health, Education, and Welfare Publication (National Institutes of Health) 78-23.

[3] Abbreviations used in this paper: αδ-dex, dextran-conjugated anti-IgD antibodies; B^{sp}, sort-purified B cells; B^e, B cell-enriched, T-depleted spleen cells; NKSN, NK cell supernatant; SN, supernatant.

Culture medium

RPMI 1640 (Biofluids, Rockville, MD) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY), L-glutamine (2 mM), 2-ME (0.05 mM), penicillin (50 μg/ml), and streptomycin (50 μg/ml) were used for culturing cells.

Reagents

αδ-dex was prepared by conjugation of $H\delta^a/1$ (monoclonal mouse IgG2b (b allotype) anti-mouse IgD (a allotype) to a high molecular weight dextran (2×10^6 m.w.) as previously described (15). Approximately 6 $H\delta^a/1$ were conjugated to each dextran molecule. LPS W, extracted from *Escherichia coli* 0111:B4, was obtained from Difco Laboratories, Inc. (Detroit, MI) and was used at 20 μg/ml in all experiments. The following mAb were purified from ascites: 2.4G2 (rat IgG2b anti-FcγRII) (16), 2C11 (hamster IgG anti-CD3) (17), S4B6.1 (rat IgG2a anti-IL-2) (18), PC-61 (rat IgG1 anti-IL-2R) (19), TRFK-5 (rat IgG1 anti-IL-5) (20), and J4–1 (rat IgG1 anti-NP) (21). 2C11 was conjugated to FITC (Calbiochem-Behring, San Diego, CA), by a standard protocol. Phycoerythrin-labeled affinity-purified goat anti-mouse IgM antibody was purchased from Southern Biotechnology Associates (Birmingham, AL). Murine rIL-5 was a kind gift from Dr. Richard Hodes, (NIH, Bethesda, MD). Human rIL-2 was a kind gift from Dr. Michael Lotze (University of Pittsburgh, Pittsburgh, PA). Percoll was obtained from Pharmacia (Piscataway, NJ).

Preparation and culture of B cells

Enriched populations of B cells were obtained from spleen cells from which T cells were eliminated by treatment with monoclonal rat IgM anti-Thy-1 (H013–4), rat IgG2b anti-CD4 (GK1.5), and rat IgG2b anti-CD8 (2.43), followed by mouse anti-rat Igκ (MAR 18.5) and complement. Cells were then fractionated into high and low density populations by centrifugation over a Percoll gradient (Pharmacia) consisting of 70, 65, 60, and 50% Percoll solutions (with densities of 1.086, 1.081, 1.074, and 1.062 g/ml, respectively). The high density cells were collected from the 70 to 65% interface and were used in all experiments. The average percentage of Ig^+ cells was 90–95% in the 70 to 65% fraction. B cells obtained in this way were referred to as "B cell-enriched" (B^e) cells. This cell population is also known to contain small numbers of NK cells, macrophages, mast cells, and cells of the granulocytic series. To remove NK cells from the B^e cell preparation we further treated B^e cells with polyclonal rabbit anti-AsGm-1 antibodies (Waco, Osaka, Japan) plus complement as follows: B^e cells (10^7 cells/ml) were incubated in "cytotoxicity medium"

(CM) (RPMI + 2.5 mM HEPES + 0.3% BSA) with anti-AsGm-1 antibody (150 μg/ml final concentration) at 4°C for 1 h. Cells were then washed once in cold CM and resuspended in CM containing a 1/10 dilution of rabbit complement (Pel-Freez, Brown Deer, Wisconsin) at 37°C for 1 h. Cells were then washed and resuspended in medium for further use. Functional assays were carried out in 96-well flat-bottomed Costar plates (Costar, Cambridge, MA). Cultured cells were incubated at 37°C in a humidified atmosphere containing 6% CO_2.

Establishment of NK cell cultures

Spleen cells from CB-17 SCID mice, obtained from NCI (Frederick, MD), were cultured in medium at 1×10^7/ml in the presence of 500 U/ml of human rIL-2. Spleen cells were first treated with anti-Thy-1, anti-CD4, and anti-CD8 + complement as a precaution against the possible presence of small numbers of T cells resulting from "leakiness" in the SCID mutation. NK cells were maintained by splitting them 1:2 into fresh medium + 500 U/ml of IL-2 every 2 to 3 days. Cells were used for experiments beginning ~7 to 10 days after establishment of culture. Such cells were monitored by flow cytometry to confirm the absence of $CD3^+$ (T) cells. NK cell cultures were re-established every month, using fresh spleen cells from SCID mice, since longer culture periods were associated with deterioration of the NK cell line. Cells derived from CB-17 mice, like those from DBA/2 and BALB/c, express H-2^d MHC class I molecules.

Cytofluorometric analysis and cell sorting

Spleen cells were stained for 30 min with FITC-labeled anti-CD3 mAb + phycoerythrin-labeled anti-IgM antibodies (final concentration of 10 μg/ml each in the presence of a 5-fold excess of anti-FcγRII mAb to prevent cytophilic antibody binding) at 10^7 cells/ml in cold clear HBSS containing 3% FBS and 50 μg/ml each of penicillin, streptomycin, and gentamicin. Cells were then washed and resuspended in staining buffer at 10^7 cells/ml in preparation for fluorescence analysis and/or cell sorting. For analysis, a FACStar Plus or FACSCAN (Becton Dickinson, Mountain View, CA) was used and 15,000 cells were collected using logarithmic amplification. Only viable cells were analyzed on the basis of their characteristic forward and side scatter profiles. Cell sorting was similarly carried out on a FACStar Plus, as well as on an Epics Elite (Coulter Corp., Hialeah, FL), and sorted cells were immediately reanalyzed to confirm their staining profile. Only sorting purities of >98% were acceptable for subsequent study. Sort-purified B cells ($mIgM^+CD3^-$) were referred to as B^{sp} cells. Non-B, non-T cells ($mIgM^-CD3^-$) and T cells ($mIgM^-CD3^+$) were also collected and macrophages were routinely eliminated, during sorting, on the basis of their characteristic forward and side scatter profile.

Quantitation of secreted IgM

IgM concentrations were measured by ELISA with Immulon 4, 96-well flat-bottomed ELISA plates (Dynatech Laboratories, Alexandria, VA), that has been described by us in detail elsewhere (22). Briefly, ELISA plates were coated with polyclonal goat anti-mouse IgM antibodies (Southern Biotechnology Associates), followed by addition of serial dilutions of samples and standards. Alkaline phosphatase-conjugated polyclonal goat anti-mouse IgM antibodies were then added, upon which a fluorescent product was generated from cleavage of 4-methylumbilliferyl phosphate (Sigma) by specifically-bound alkaline phosphatase-conjugated antibodies. Fluorescence was measured on a 3M FluoroFAST 96 fluorometer (Mountain View, CA) and fluorescence units were converted to Ig concentrations by extrapolation from standard curves determined in each assay by using purified myeloma IgM of known concentration. IgM measurements showed no significant cross-reactivity or interference from the presence of other isotypes (IgD, IgG3, IgG1, IgG2b, IgG2a, IgE, and IgA).

Results

Small B^{sp} cells fail to secrete Ig in response to αδ-dex + IL-2

We previously demonstrated that IL-2 or IL-5 stimulated small, T-depleted, B cell-enriched spleen cells (B^e cells) to secrete Ig in vitro in the presence of αδ-dex (6). To determine whether these cytokines acted directly on the B cell to stimulate maturation to Ig production, we employed sort-purified (B^{sp}) cells which were >98% mIg^+ (Fig. 1). B^{sp} cells were cultured with αδ-dex in the presence of IL-2 or IL-5, or with LPS, and the IgM secretory responses were compared with similarly treated B^e cells. Both B^e and B^{sp} cells secreted comparable amounts of IgM in the presence of αδ-dex + IL-5 or LPS (Table I). By contrast, IgM secretion by B^{sp} cells in response to αδ-dex and IL-2 was only marginally greater than that seen in control cultures which were activated with αδ-dex or IL-2 alone. Similar amounts of Ig were secreted, in response to αδ-dex plus IL-2, by B^e cells stained with anti-IgM but not purified by cell sorting vs unstained B^e cells (data not shown). This ruled out a role for anti-IgM staining in the failure of B^{sp} cells to secrete Ig under these conditions. This suggested that the IL-2-stimulated Ig secretory responses of the T-depleted B cell population depended upon the presence of non-B, non-T cells. IgM secretion by αδ-dex-activated B^e cells in the presence of IL-2 or IL-5 constituted ~95% of the secreted Ig isotypes (ref. 9; data not shown), and thus approximated the total Ig secretory response.

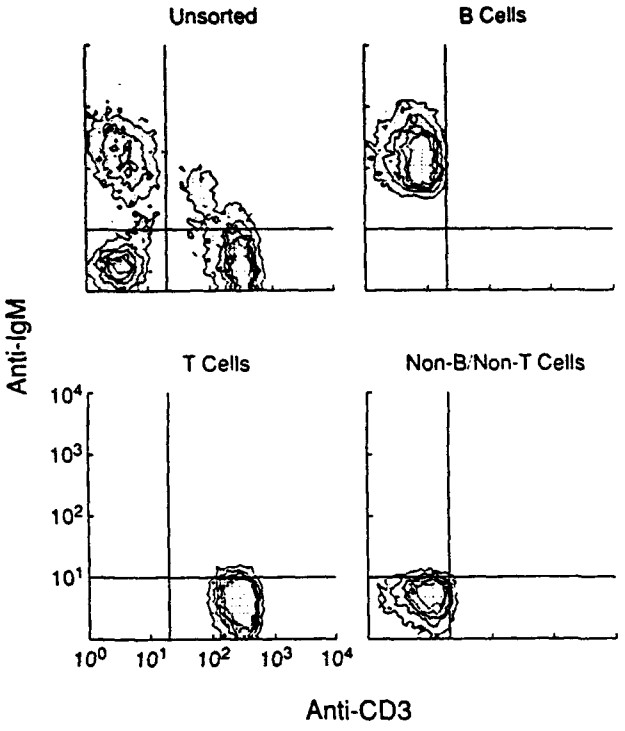

FIGURE 1. Flow cytometric analysis and cell sorting of splenic B, T, and non-B, non-T cells. Spleen cells were stained with FITC-anti-CD3 + PE-anti-IgM (*top left*, "Unsorted"). B cells ($CD3^-IgM^+$), T cells ($CD3^+IgM^-$), and non-B, non-T cells ($CD3^-IgM^-$) were sorted to high purity (>98%). Contour graphs represent sorted cells which were analyzed immediately after sorting to assess purity.

Table I
Sort-purified B cells fail to secrete IgM in response to αδ-dex + IL-2[a]

	IgM Secretion (ng/ml)	
	B^e	B^{sp}
Med	<135	<135
αδ-dex	540	170
IL-2	<135	<135
αδ-dex + IL-2	21,250	850
αδ-dex + IL-5	33,750	17,250
LPS	131,250	112,500

[a] B^e (B cell-enriched; ~90% B) and B^{sp} (sort-purified; >98% B) cells were cultured at 1.25×10^5 cells/ml in the presence of αδ-dex (3 ng/ml), IL-2 (150 U/ml), IL-5 (150 U/ml) and/or LPS (20 μg/ml). Six days later culture SN was removed for measurement of IgM concentrations by ELISA.

To more directly demonstrate a requirement for non-B, non-T (IgM^-CD3^-) cells in the αδ-dex + IL-2-induced Ig secretory response of B^e cells, we obtained B^{sp} and non-B, non-T cells by electronic cell sorting of a population of small B^e cells. Macrophages were excluded during cell sorting on the basis of their characteristic forward and side scatter profile. As before, αδ-dex + IL-2 induced a large Ig secretory response by small B^e, but not B^{sp} cells (Fig. 2). However, the addition of non-B, non-T cells to αδ-dex + IL-2-induced B^{sp} cells led to a 10-fold increase in Ig secretion which was roughly comparable to that observed in cultures of αδ-dex + IL-2-stimulated B^e cells in the presence or absence of non-B, non-T cells. Non-B, non-T cells by themselves failed to stimulate small B^{sp} or B^e cells in the absence of αδ-dex + IL-2 (data not shown). Thus, the αδ-dex + IL-2-stimulated Ig secretory response of B^{sp} cells required the presence of one or more non-T cell types.

Treatment of B^e cells with anti-AsGm-1 antibodies plus complement selectively inhibits Ig secretion in response to αδ-dex plus IL-2

Since the non-B, non-T cell splenic population is known to contain NK cells, and since NK cells are capable of releasing cytokines upon activation, we tested whether such cells played a key role in Ig induction by B^e cells in response to αδ-dex plus IL-2. Thus, B^e cells were depleted of NK cells by treatment with anti-AsGm-1 antibodies plus complement and stimulated with either αδ-dex plus IL-2 or αδ-dex plus IL-5, as a control. As indicated in Table I, IL-5-mediated induction of Ig secretion occurs in αδ-dex-activated B^{sp} cells and hence is not dependent on another cell type. Anti-AsGm-1 antibody-treated B^e cells showed a marked reduction in Ig secretion in response to αδ-dex plus IL-2 but not after activation with αδ-dex plus IL-5 (Fig. 3). This strongly suggested that splenic NK cells played an inductive role in the αδ-dex plus IL-2-mediated Ig secretory response of B^e cells.

NK cells stimulate αδ-dex + IL-2-induced B^{sp} cells to secrete Ig

To further explore a role for NK cells in stimulating Ig secretion by αδ-dex plus IL-2-activated B cells, we established a pure NK cell culture by culturing, in IL-2, spleen cells from 6- to 8-wk-old SCID mice, which lack T and B cells (24). Since the SCID mutation can be "leaky," especially in older mice (25), we first treated SCID spleen cells with anti-Thy-1, anti-CD4, and anti-CD8 antibodies plus complement and then assessed the purity of our resulting IL-2-induced NK cell population 7 days later by flow cytometric analysis of their cell surface phenotype (Fig. 4). That we were dealing with a pure population of NK cells was supported by the observation that essentially all cells were $IgM^-CD3^-CD4^-CD8^-Thy\text{-}1^+AsGm\text{-}1^+$. In vitro-generated NK cells as well as freshly explanted splenic T (IgM^-CD3^+) and non-B, non-T (IgM^-CD3^-) cells, obtained by electronic cell sorting, were co-cultured with αδ-dex-activated B^{sp} cells in the presence of IL-1 + IL-2, and IgM production was measured 6 days later (Fig. 5). IL-1 was included in this and subsequent experiments based upon our observation that the level of the αδ-dex + IL-2-mediated Ig secretory response of B^e cells varied among different experiments, and that the additional presence of

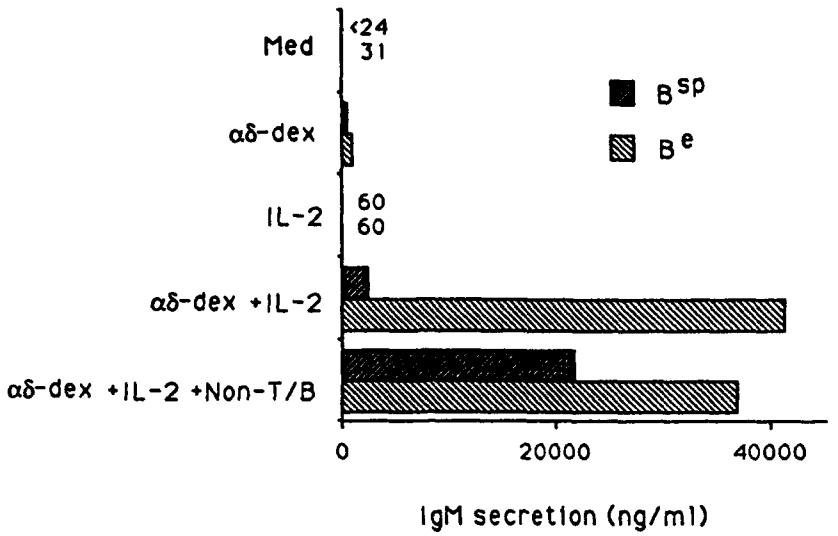

FIGURE 2. Non-B, non-T cells restore the IgM secretory response of sort-purified B cells in response to αδ-dex + IL-2. B^e (B cell-enriched; ~90% B) and B^{sp} (sort-purified; >98% B) were cultured at 1.25 × 10^5 cells/ml in the presence of αδ-dex (3 ng/ml) + IL-2 (150 U/ml) in the presence or absence of sort-purified splenic non-B, non-T cells (10% final cell concentration). Six days later, culture SN was removed for measurement of IgM concentrations by ELISA.

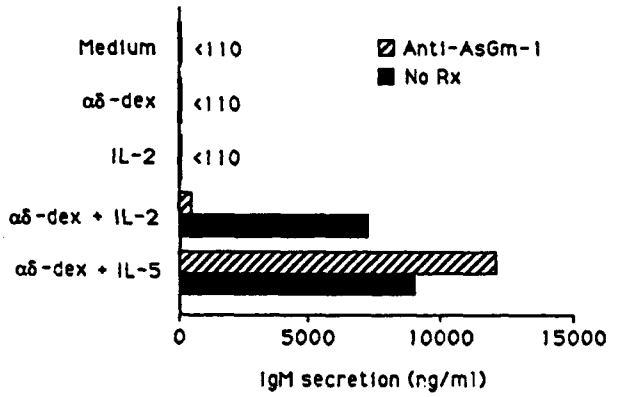

FIGURE 3. Treatment of B^e cells with anti-AsGm-1 antibodies plus complement selectively inhibits Ig secretion in response to αδ-dex plus IL-2. B^e cells were depleted of NK cells by in vitro treatment with anti-AsGm-1 antibodies plus complement as described in *Materials and Methods* and compared with B^e cells receiving no further treatment. Cells were stimulated at 1.25 × 10^5/ml with αδ-dex (3 ng/ml) and/or IL-2 (150 U/ml) or αδ-dex plus IL-5 (150 U/ml). Culture SN was removed 6 days after initiation of culture for determination of secreted IgM concentrations by ELISA.

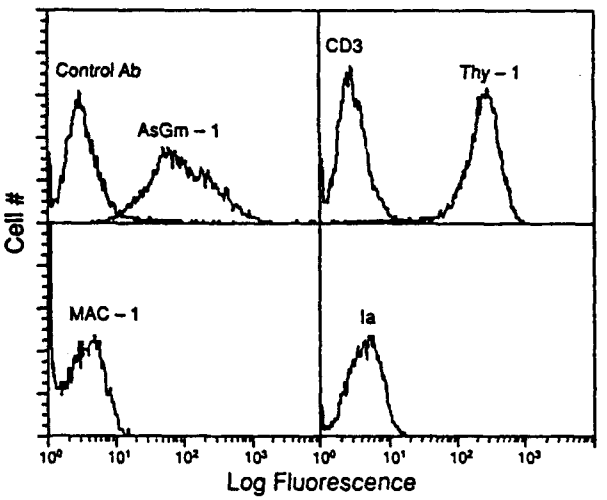

FIGURE 4. Flow cytometric analysis of in vitro-generated NK cells. In vitro-generated NK cells were harvested 7 days after initiation of spleen cell cultures in the presence of IL-2 (see *Materials and Methods*). Cells were stained with antibodies to the indicated cell surface proteins. FITC-Rat IgG2b anti-mouse Thy-1.2 mAb (30H12) (ref. 38), FITC-mouse IgG2a anti-mouse Ia^d (MKD6) (ref. 39), rat IgG anti-mouse MAC-1 (M1/70) (ref. 40), control Ab (FITC-rat IgG anti-mouse IgG2a, Zymed Laboratories, So. San Francisco, CA).

IL-1 typically assured a maximal response (data not shown). The addition of NK cells to αδ-dex + IL-1 + IL-2-activated B^{sp} cells led to a strong induction of IgM secretion that ranged from between 10- and 20-fold in five separate experiments. This response was essentially comparable to that seen when freshly explanted splenic non-B, non-T cells were used instead of cells from an IL-2-induced NK cell culture (Fig. 5). Small B^{sp} cells failed to secrete Ig in the presence of NK cells unless both αδ-dex and IL-2 were present (data not shown). By contrast, T cells showed no Ig-inducing activity in this assay. Varying the ratio of NK cells to αδ-dex + IL-2-activated B^{sp} cells (1.0 to 80% NK:B) indicated that the presence of 10% NK cells led to maximal induction of Ig secretion, with <1% NK cells generally being ineffective (Fig. 6).

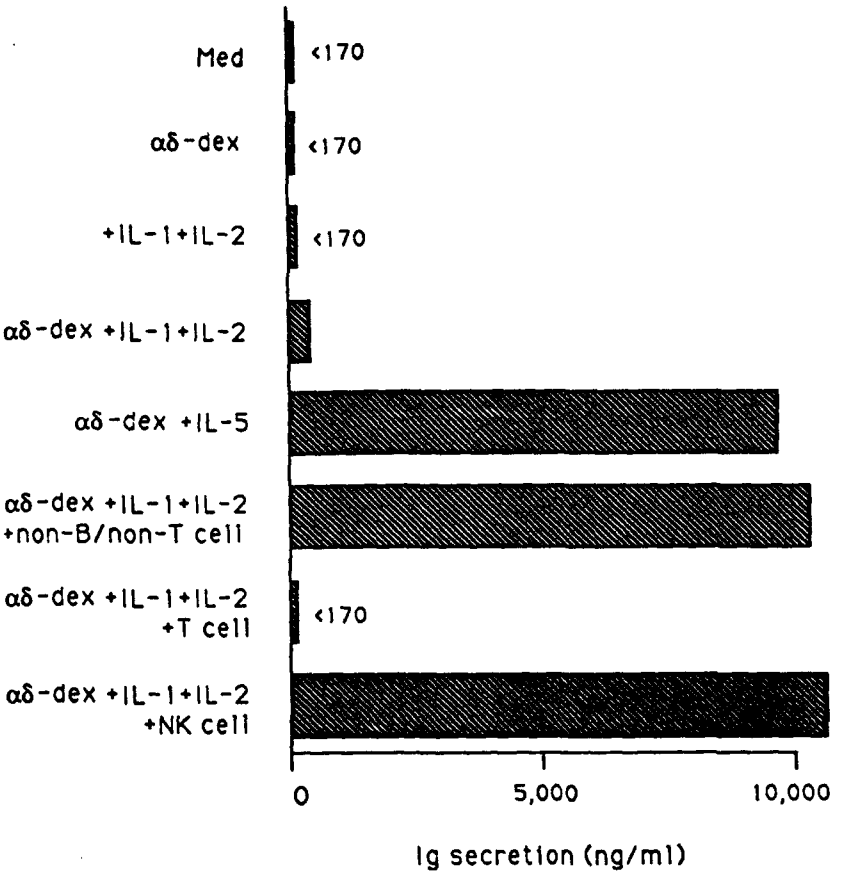

FIGURE 5. NK cells stimulate sort-purified B cells to secrete IgM in response to αδ-dex + IL-2. B^{sp} cells were cultured at 1.25×10^5/ml with IL-1 (150 U/ml) + IL-2 (150 U/ml) in the presence or absence of sort-purified T cells (10% final concentration) or non-B, non-T cells (10% final concentration) or in vitro-generated NK cells (10% final concentration). IL-5 was used at 150 U/ml. Six days later culture SN were removed for measurement of IgM concentrations by ELISA.

IL-2-induced NKSN can replace NK cells for induction of Ig secretion by αδ-dex-activated B^{sp} cells

We next wished to determine whether the αδ-dex + IL-2-induced Ig secretory response of B^{sp} cells required co-culture of NK cells and/or was mediated by the release of an NK cell-derived maturation factor. Thus, cells from an established IL-2-activated NK cell culture were washed and recultured in the presence of IL-2, alone, for 48 h, upon which a cell-free supernatant (NKSN) was prepared. The addition of IL-2-containing NKSN to cultures of αδ-dex-activated B^{sp} cells led to a strong induction of Ig secretion, compared with B^{sp} cells activated with αδ-dex + IL-2 alone (Table II). The induction of Ig secretion was higher at 25% v/v NKSN than at 10%, even when cultures were supplemented with additional IL-2. These findings indicate that co-culture of B and activated NK cells is not required for Ig induction.

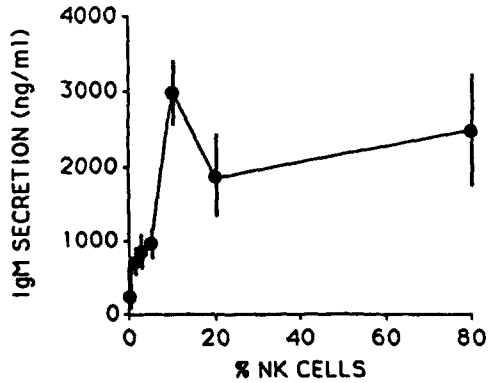

FIGURE 6. Titration of NK cell numbers for determination of optimal induction of IgM secretion of B^{sp} cells in response to αδ-dex + IL-1 + IL-2. B^{sp} cells were cultured at 1.25×10^5 cells/ml in the presence of IL-1 (150 U/ml) + IL-2 (150 U/ml) with varying concentrations of NK cells (1.25×10^4 [1.0%] to 1.0×10^5 cells/ml [80%]). Culture SN was removed 6 days later for measurement of IgM concentrations by ELISA.

B cells also require IL-2 for NSKN-induced Ig secretion

We observed that the established NK cell culture was critically dependent upon the presence of IL-2, since removal of IL-2 from culture led to over 80% loss of viability of NK cells within 24 h. To determine whether B cells also required IL-2 for induction of Ig secretion we added anti-IL-2 and anti-IL-2R mAb to B^{sp} cultures stimulated with αδ-dex + IL-2-containing NKSN (Fig. 7). Compared with a control mAb, anti-IL-2 + anti-IL-2R strongly suppressed the Ig secretory response induced by αδ-dex + NKSN. By contrast, the addition of anti-IL-2 + anti-IL-2R to B^{sp} cul-

Table II
NKSN stimulates sort-purified B cells to secrete IgM in the presence of αδ-dex + IL-1 + IL-2[a]

	IgM Secretion (ng/ml)	
	B^e	B^{sp}
Med	26	<5
αδ-dex	160	120
IL-1/IL-2	54	<5
αδ-dex + IL-1/IL-2	7,500	220
αδ-dex + IL-5	23,100	15,000
NKSN		16
αδ-dex + IL-1/IL-2 + NKSN		8,750

[a] B^e (B cell-enriched; ~90% B) and B^{sp} (sort-purified; >98% B) cells were cultured at 1.25×10^5 cells/ml in the presence of αδ-dex (3 ng/ml), IL-1 (150 U/ml) + IL-2 (150 U/ml), IL-5 (150 U/ml), and/or NKSN (25% v/v). Six days later culture supernatants were removed for measurement of IgM concentrations by ELISA.

tures activated with αδ-dex + IL-5 had no effect. Thus, for induction of Ig secretion, IL-2 is required at the level of the B cell, as well as of the NK cell.

IL-5 is not required for IgM secretion by αδ-dex + IL-1 + IL-2-activated B cells in response to non-B, non-T cells or NKSN

Since αδ-dex-activated, sort-purified, B cells secrete IgM in response to IL-5, we wished to determine whether endogenous IL-5 played a role in the IgM secretory responses of αδ-dex + IL-1 + IL-2-activated B cells to non-B, non-T cells or NKSN. The addition of a neutralizing anti-IL-5 mAb (TRFK-5) to cultures of B^e cells stimulated by αδ-dex + IL-2 (Fig. 8*A*) or B^{sp} cells activated with αδ-dex + NKSN (Fig. 8*B*) failed to inhibit the Ig secretory response, indicating that IL-2 did not work through induction of IL-5.

Discussion

This study demonstrates that splenic NK cells can, in the presence of IL-2, stimulate Ig secretion by small, resting B cells that have been activated by cross-linking of their mIg in a TI-2-like fashion. NK cells may induce Ig secretion through release of an as yet unidentified B cell maturation factor, since direct physical contact between B and activated NK cells is not necessary.

These studies extend the findings reported in the human system describing the ability of NK cells to stimulate B cell maturation to Ig secretion (26–28). Our data indicating a role for NK cells in stimulating Ig secretion are also consistent with a recent report by Yuan et al. (29) demonstrating the ability of murine NK cells to stimulate small, resting murine B cells to secrete Ig in vitro in the presence of IL-2 and IL-5. Our data, utilizing αδ-dex as a key B cell activating signal, establish an in vitro animal model for exploring the role of NK cells in stimulating T cell-independent pathways of immunity. Analogous to our findings, these studies have also demonstrated the ability of NK cells to release a soluble B cell maturation factor(s) which stimulates B cells to secrete Ig (26, 27, 29). Thus, NK cells could, in theory, play a particularly important role in humoral immune responses to bacteria, which contain within their cell walls TI-2, polysaccharide, Ag, and perhaps substances that stimulate NK cells. In this regard, it has been demonstrated that T and B cell deficient SCID mice that have been infected with the bacterium *Listeria monocytogenes* contain NK cells that have been induced to secrete IFN-γ (11, 12). Although IL-2, which was required for induction of Ig synthesis, is considered primarily a T cell-derived cytokine, it has been demonstrated previously that both B (30–32) and NK cells (33–35) can release IL-2

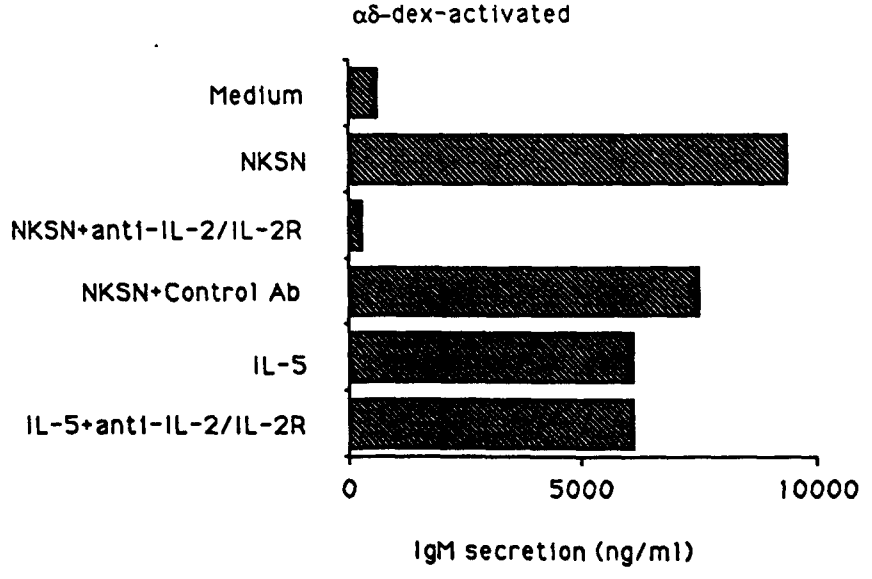

FIGURE 7. Induction of IgM secretion by B^{sp} cells in response to αδ-dex + NKSN requires IL-2. B^{sp} cells were cultured at 1.25×10^5 cells/ml in the presence of αδ-dex + NKSN (10% v/v) or IL-5 (150 U/ml). Anti-IL-2 (30 μg/ml)+ anti-IL-2R (30 μg/ml) or a control mAb (60 μg/ml) were added to selected wells. Culture SN was removed 6 days later for measurement of IgM concentrations by ELISA.

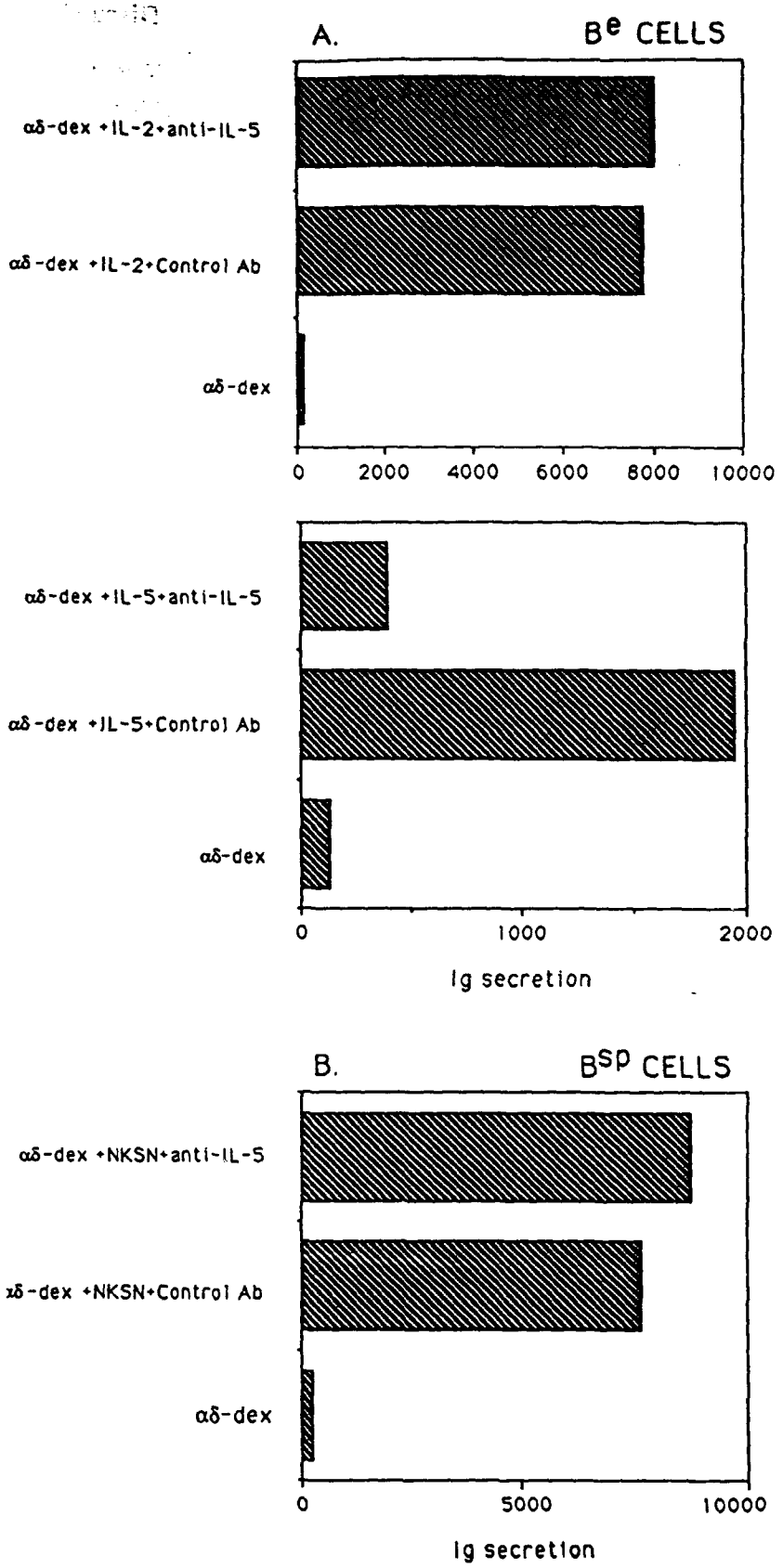

FIGURE 8. IL-5 is not critical for induction of IgM secretion in responses of αδ-dex + IL-2-activated B cells to non-B, non-T cells or NKSN. *A*, B^e cells were cultured at 1.25×10^5 cells/ml in the presence of αδ-dex +/- IL-2 (150 U/ml) or IL-5 (150 U/ml) with anti-IL-5 mAb (30 μg/ml) or an isotype-matched control mAb (30 μg/ml). Six days later culture SN was removed for measurement of IgM concentrations by *ELISA*. *B*, B^{sp} cells were cultured at 1.25×10^5 cells/ml in the presence of αδ-dex ± NKSN with anti-IL-5 (30 μg/ml) or an isotype-matched control mAb (30 μg/ml). Six days later culture SN was removed for measurement of IgM concentrations by *ELISA*.

upon appropriate activation. Thus, TI pathways of activation can induce the release of IL-2, which would play an important role in regulating B cell responses.

NK cells could also play an important role in Ig isotype regulation through their release of IFN-γ, which we previously reported to be a switch factor for both IgG3 (9) and IgG2a (10). Thus, it has been demonstrated that in vitro co-culture of large murine B cells with purified NK cells induces the release of IFN-γ (36). It has further been shown that NK cells can provide a source of IFN-γ that stimulates IgG2a secretion by LPS-activated murine B cells in vitro (13, 14). In this regard, we have recently observed that the addition of anti-IFN-γ mAb to cultures of αδ-dex + IL-2-stimulated B^c cells selectively inhibits the secretion of IgG2a (C. M. Snapper, unpublished observations).

NK cells have been shown to suppress humoral immunity and it is widely accepted that NK cells can exhibit cytotoxicity for selected target cells (37). Although the basis for NK cell down-modulation of humoral immune responses is unknown, it could reflect either the release of IFN-γ, which under certain instances inhibits Ig secretion, and/or direct killing of the B cell. Hence, NK cells, like T cells, exhibit both helper and suppressor activities for humoral immune responses. Although NK cells are known to possess both phenotypic and functional heterogeneity, the basis for the reported dichotomy in their regulation of antibody production and the mechanism(s) which determine the predominance of one activity over another are unknown. This idea has support from other studies showing that either anti-LFA-1 or anti-CD2 antibody abrogates cytotoxicity by inhibiting NK cell interaction with susceptible targets, but does not inhibit their ability to mediate B cell help for antibody secretion (28). This suggests that these two NK cell-mediated processes can be distinguished mechanistically.

While we have not, as yet, identified the nature of the B cell maturation factor released by murine NK cells in the presence of IL-2, preliminary studies utilizing recombinant cytokines and neutralizing anti-cytokine mAb have ruled out a critical role for IFN-γ, IFN-α/β, TNF-α, GM-CSF, TGF-β, IL-1, IL-3, IL-4, IL-5, and IL-10 (data not shown). Studies in the human have also ruled out a critical role for IL-4, IFN-β1, IFN-γ, GM-CSF, TNF-α, or low molecular weight BCGF (27). Furthermore, IL-6, which has been described to be an important cytokine both in the human and murine system for inducing Ig secretion, could not account for the full maturation activity of NKSN on B^{sp} cells. These preliminary data suggest that the NK-derived maturation factor may be a novel cytokine. The presence, however, of IL-2 is necessary both for NK cell and B cell stimulation. Thus, even in the presence of an IL-2-generated, NK cell-derived, maturation factor, anti-IL-2 and anti-IL-2R antibodies inhibited Ig secretion by sort-purified B cells. Since B^{sp} cells were from 98 to 99% pure, we cannot formally rule out the possibility that the IL-2 requirement apparent at the level of the B cell was in fact at the level of a small contaminating cell population in the B^{sp} cell preparation.

In summary, we have described the ability of murine NK cells to stimulate Ig secretion by B cells activated in a TI-2-like manner. The identification of the NK cell-derived B cell maturation factor(s) and the ability to identify a role for NK cells in stimulating Ig secretion during immune responses in vivo may help to clarify some of the parameters that regulate TI pathways of humoral immunity.

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